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Biological Activity of Plant Essential Oils against *Fusarium circinatum*†

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Abstract: The fungus *Fusarium circinatum* causes pitch canker in susceptible *Pinus* spp. and Douglas fir. Infection promotes damping-off, resin-streaming cankers on main stems and lateral branches, shoot dieback, needle chlorosis or discoloration, cone death, and increased tree mortality. Essential oils (EOs) can provide eco-friendly alternatives for chemical fungicides. The present work reviewed the available literature on EOs tested against *F. circinatum*. The 62 tested EOs were extracted mainly from plants belonging to the families Myrtaceae, Compositae and Apiaceae. The highest activities were reported for *Cinnamomum verum*, *Cymbopogon citratus*, *Foeniculum vulgare*, *Syzygium aromaticum* and *Thymus vulgaris* EOs. A higher investment on the screening of natural compounds as eco-friendly fungicides against pitch canker is necessary to promote more sustainable disease control measures.

Keywords: essential oil; forest management; fungicide; *Fusarium circinatum*; phytosanitary measures; *Pinus*; pitch canker; tree disease

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1. Introduction

Pitch canker is a highly damaging pine pathology caused by the fungus *Fusarium circinatum* Nirenberg & O'Donnell (teleomorph = *Gibberella circinata* Nirenberg & O'Donnell). *F. circinatum* is known to infect pine trees (genus *Pinus*) and the Douglas fir (*Pseudotsuga menziesii*) and is distributed throughout the globe. The disease is believed to have originated in Mexico and later spread to several parts of the American continent, having been detected in the coastal south-eastern states of the USA and in Haiti and Chile. Around the world, it has been identified in Japan and Korea, in South Africa and more recently in Europe, in Italy, France, Spain and Portugal [1,2]. Although all pines are believed to be susceptible by this disease, the symptomatology of pine pitch canker was found to be generally highly dependent on the host age and species, and on biotic and abiotic conditions, such as temperature, humidity, soil properties, insect pathogens, etc., exhibiting different degrees of severity according to the type and location of the pine culture [1,3]. Damage to Douglas fir is generally less severe than to *Pinus* spp., but firs can contribute to the spread of the disease by becoming dissemination points. This fungal phytopathogen does not usually penetrate intact healthy tissues, so infection generally occurs in wounded areas, namely through environmental, insect or human action. Spread of the disease from infected trees to susceptible healthy pines is usually dependent on water (e.g., rain), wind or vectored by bark beetles, circulating between tree branches and among trees, although dissemination to new areas is usually slower. Pine seeds are also believed to become infected, having a substantial role in accelerating spread to new areas. A much higher incidence of the disease is detected in pine nurseries and plantations than on wild pine stands [1]. In contaminated pines, the fungal pathogen induces damping-off and resin-streaming cankers on main stems and lateral branches, as the infected areas begin to exude increased amounts of resin and the tissue becomes necrotic, after the exuded resin saturates the xylem tissue. Needle chlorosis or discoloration is also reported and, as the disease progresses, the drooping dead needles become drenched in resin and adhere for extended periods of time. Severe disease symptoms include progressive shoot dieback, cone death, and ultimately increased tree mortality [4].

2. Control strategies for pitch canker

Current management strategies used in pine nurseries and plantations rely on conservative measures, such as the elimination of diseased plants and plant material, and on adequate hygiene and phytosanitary measures to prevent unwanted accumulation of water or an increase in insect populations. Additionally, biocidal treatments are applied to the irrigation water and the surfaces sterilized. The use of insecticides, to control insect populations, and fungicides, to prevent fungal infections, is frequent. Nevertheless, many active biocides have been recently withdrawn in Europe, which prompted research on natural compounds, such as essential oils (EOs) [5].

2.1 Essential oils as biopesticides

EOs are defined as "a product obtained from natural raw material of plant origin, by steam distillation, by mechanical processes from the epicarp of citrus fruits, or by dry distillation, after separation of the aqueous phase, if any, by physical processes", by the International Organization for Standardization (ISO 9235). They are commonly obtained in the form of a concentrated hydrophobic liquid, at room temperature, containing volatile aroma compounds [6,7]. The chemical composition of most EOs is dominated by mono- and sesquiterpenes and some phenolic compounds, such as phenylpropanoids, although other groups of compounds can also occur in relevant amounts. Despite being commonly used in the food, perfumery and pharmaceutical industries, EOs have also been reported as successful biologically active substances, showing high anti-microbial, insecticidal, acaricidal, herbicidal, nematicidal and strong fungicidal activities [7–11]. Over 20 000 studies were reported to deal with the biological activities of EOs, being ca. 25% performed on the antioxidant activity, 12% on antimicrobial activities, and 11% on insecticidal and insect repellent activities [12]. These natural products are a good source of environmentally safer biopesticides or of model compounds for the synthesis of easily biodegradable derivatives, that show negligible plant toxicity as well as safety for humans [13,14]. In addition to not accumulating in the environment, EOs can display diverse biological activities since they are composed of multiple phytochemicals in different amounts. In these complex mixtures, biological activity frequently results from the biocidal EO phytochemicals combined with EO compounds that present no direct activity on the plant pest, yet are capable of influencing resorption, rate of reaction and bioavailability of active phytochemicals. Additionally, EO compounds can display additive, synergistic or antagonistic interactions, given the combined effect be equal, exceed or be less than to the sum of the individual effects [7,15–17]. EOs have also less strict regulatory approval mechanisms for their exploration, due to a long history of use [18].

The present work reviewed the available literature on EOs tested against the pitch canker fungus and identified the plant sources with the most successful fungicidal properties. Summarizing information on biocidal EOs against *F. circinatum* enables an easily accessible comparative analysis of the compositions and activities of natural fungicides and contributes for the establishment of more sustainable disease control strategies.

3. Bibliographic data

Research was performed with Web of Science (WoS) search engine (last accessed May 2021), in all available databases, on published works using the topics "*Fusarium circinatum*" or "pitch canker" and "essential oil"

The reports retrieved were published in journals specialized in the scientific fields of Agricultural engineering, Agronomy, Biochemistry and molecular biology, Applied chemistry, Entomology, Food science and technology, Forestry and Physiology. These reports were cited 147 times by a total of 138 works, with an average of 36.75 citations *per* report. The cumulative number of citations has increased steadily, suggesting a constant research interest in this subject (Figure 1).

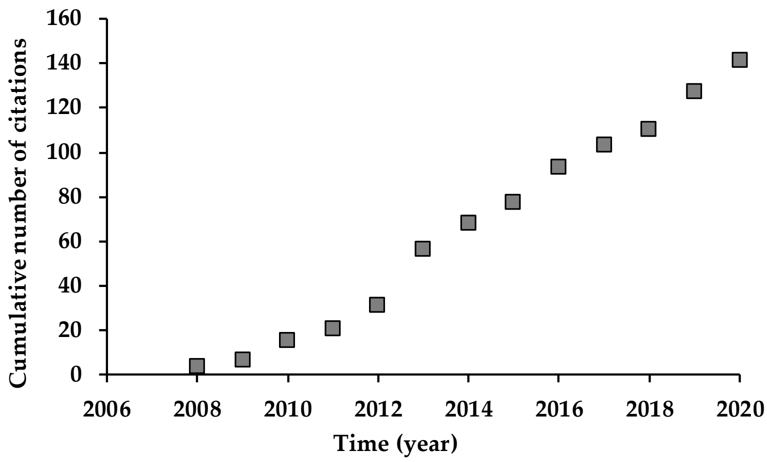
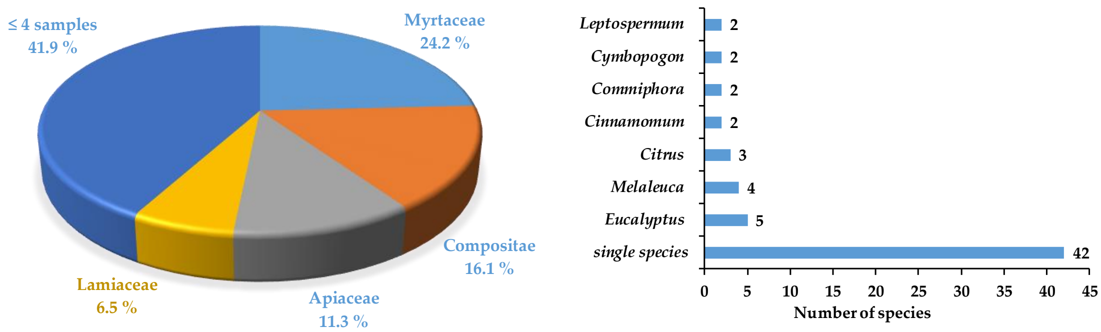


Figure 1. Cumulative number of citations on works reporting the activity of essential oils against pitch canker fungus (*Fusarium circinatum*).

4. EO tested against *F. circinatum*

A total of 62 EOs from 61 plant species was tested against the pitch canker fungus. The reported EOs were extracted from plants belonging to 20 families, with the families Myrtaceae (24 %), Compositae (16 %), Apiaceae (11 %) and Lamiaceae (7 %) being the most represented (Figure 2a). The plant sources used were mostly from several different genus, however the genus *Eucalyptus*, *Malaleuca* and *Citrus* were more frequently assayed (Figure 2b).



(**a**) (**b**)

Figure 2. Plants sources of the essential oils tested against *Fusarium circinatum*, compiled according to (**a**) family (% of samples) and (**b**) genus (number of reported species).

3.1. Fungicidal activity of EOs

Parameters of activity against the pitch canker fungus were mainly reported for the most successful EOs. Complete fungicidal activity (100%) was identified for the EOs of *Cinnamomum verum* and *Foeniculum vulgare* and complete fungistatic activity for *Syzygium aromaticum*, applied at 10 %, 15 % and 50 %, respectively, although phytotoxic activities against *Pinus radiata* were simultaneously reported [4]. *In vitro* mycelium growth inhibition (MIC) values below 500 µL/L were reported for the EOs extracted from *Cymbopogon citratus*, *S. aromaticum* and *Thymus vulgaris*. Additionally, considerable variability in MIC values was discovered for different fungal strains, suggesting a high genetic influence on EO activity [19]. Two studies reported EO compositions and tested the main compounds *in vitro* against *F. circinatum*. Moderate activities were detected for the phenylpropanoid cinnamyl aldehyde (29 % growth inhibition), the main compound of *Liquidambar orientalis* EO [20] and for the monoterpenoids citronellol (38 % growth inhibition) and neral (32 % growth inhibition), both present on the EOs of *Corymbia citriodora* and *Leptospermum petersonii* [5]. Surprisingly, no activity was reported for the phenylpropanoids cinnamyl alcohol and hydrocinnamyl alcohol as well as for the monoterpenoids citronellal, geraniol and geranial (*trans* isomer of neral), which might indicate that the previous fungicidal EO compounds may have specific molecular targets.

4. Conclusion

The study of EOs with fungicidal activity against *F. circinatum* is still in a preliminary stage and requires additional bioassays as well as a more extensive screening to identify highly active EOs or groups of fungicidal volatiles capable of integrating more sustainable disease control strategies.

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